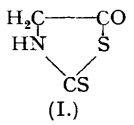
125. *Studies in the Azole Series. Part XXVII. A New Method of Peptide Synthesis : Glycyl Peptides.*

By A. H. Cook and A. L. Levy.

2-Thio-5-thiazolidone (I) is a useful reagent for the addition of a glycyl residue to the ester of an α-amino-acid or peptide in a single operation. Coupling takes place in the presence of a tertiary base and the product is released by acidification. In this way, for example, the polyglycine esters up to pentaglycine ethyl ester hydrochloride (III) have been prepared stepwise from glycine ester, in good yields at each stage. α-Amino-acid or peptide esters are very suitable for partition chromatography on paper, and may be revealed by the use of ninhydrin in the usual way.

In Part XIX (Billimoria and Cook, *J.*, 1949, 2323) anhydrocarboxyglycine was shown to be too reactive to allow a useful synthesis of glycylglycine ester. In Part XXV, however (*J.*, 1950, 637) a method for the controlled preparation of glycine amides from the dithio-analogue, 2-thio-5-thiazolidone (I), was established in principle, using morpholine as a model base. In the present paper, the application of this reaction to α-amino-esters is described and shown to provide a ready method for the synthesis of glycyl peptides.



(I.)

$$+ 2\,NH_2\cdot CH_2\cdot CO_2Et \longrightarrow \underset{NH\cdot CS_2H,NH_2\cdot CH_2\cdot CO_2Et}{CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et}$$

(II.)

$$\xrightarrow{HCl} \underset{NH_2,HCl}{CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et} + CS_2 + HCl,NH_2\cdot CH_2\cdot CO_2Et$$

When 2-thio-5-thiazolidone (I) was treated with two equivalents of glycine ethyl ester in ethyl acetate, the dithiocarbamate (II) separated as an uncrystallisable oil. In chloroform, however, a clear solution resulted and, when this was treated directly with dry hydrogen chloride and evaporated, a mixture of glycine ester hydrochloride and glycylglycine ester hydrochloride was obtained. A better procedure was to conduct the initial condensation in ethanol, acidification of the clear solution with ethanolic hydrogen chloride causing separation of the crude peptide ester hydrochloride in 78% yield. This was freed from glycine ester hydrochloride by one crystallisation from ethanol, the yield of pure material being then 50%.

Glycylglycine ethyl ester (two equivalents) was added to one equivalent of (I) in ethanol, followed almost immediately by two equivalents of ethanolic hydrogen chloride, which caused rapid separation of almost pure triglycine ester hydrochloride in 96% yield, the filtrate yielding crystals of the dipeptide ester hydrochloride on storage. Treatment of (I) with one equivalent only of glycine ester gave a poor yield of impure dipeptide. However, (I) reacted smoothly with a mixture of one equivalent each of glycine ester and triethylamine in chloroform, and acidification then caused pure glycylglycine ester hydrochloride to separate in 57% yield, triethylamine hydrochloride being soluble in chloroform.

$$(I) + \underset{NEt_3}{NH_2\cdot CH_2\cdot CO_2Et} \longrightarrow \underset{NH\cdot CS_2H,NEt_3}{CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et} \xrightarrow{HCl} \underset{NH_2,HCl}{CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et} + CS_2 + NHEt_3Cl$$

The peptide ester hydrochlorides may conveniently be converted into the bases *in situ* by treating the thiothiazolidone directly with an amino-ester hydrochloride and two equivalents of triethylamine. Thus, (I) in ethanol gave an 86% yield of the tripeptide ester hydrochloride, the yield of glycylglycine ester by this procedure being 70%. This general method was used in

$$(I) + \underset{2NEt_3}{HCl,NH_2\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et} \xrightarrow{2\,HCl} \underset{NH_2,HCl}{CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Et} + CS_2 + 2NHEt_3Cl$$

subsequent experiments. When it was applied to the above tripeptide ester hydrochloride, tetraglycine ethyl ester hydrochloride was obtained in a yield of 88%. The latter gave a strong biuret reaction, unlike the starting material and lower peptides. This fact is responsible for the trivial name " biuret base " given by Curtius (*Ber.*, 1883, **16**, 755; 1904, **37**, 1286) to the tetrapeptide ester, which he prepared by keeping glycine ester for several weeks in ahydrous ether. Curtius recorded for the hydrochloride m. p. 192—193°, whereas Fischer (*Ber.*, 1904, **37**, 2504), who prepared the compound by esterification of tetraglycine, gave a value of 212—214°. We have confirmed the latter figure and shown by paper chromatography that the material prepared

by tetramerising glycine ester contains a small proportion of penta- and hexa-peptides which depress the melting point.

$$HCl,NH_2\cdot[CH_2\cdot CO\cdot NH]_4\cdot CH_2\cdot CO_2Et \qquad (III.)$$

Conversion of " biuret base " hydrochloride into the *pentapeptide ethyl ester hydrochloride* (III) in a yield of 85% was achieved in a similar fashion, except that 92% methanol was required to dissolve the more complex starting material. It has previously been demonstrated with the model tripeptide synthesis, that the method was still applicable in the presence of 75% ethanol, though the yield dropped to 64% as compared with 86% in the anhydrous solvent, a result which was not unexpected in view of the known fission of *N*-dithiocarboxyglycine amides by aqueous acids.

The general procedure was applicable also to amino-esters other than glycine, in both their optically active and their racemic forms. Thus when L-tyrosine methyl ester was treated with (I) and triethylamine in chloroform, addition of dry hydrogen chloride gave *glycyl-L-tyrosine methyl ester hydrochloride* in a yield of 50%. This yield dropped to 20% when two equivalents of triethylamine were present, but increased slightly to 57% with 1·5 equivalents each of (I) and triethylamine. Similarly, DL-phenylalanine ester gave glycyl-DL-phenylalanine methyl ester hydrochloride in a yield of 30% which was not improved by several variations in the reaction conditions. When 2-thio-4-phenyl-5-thiazolidone (Billimoria and Cook, *loc. cit.*) became available, it was condensed with glycylglycine ester hydrochloride, giving 37% of *α-phenyl-*

$$\begin{matrix} CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO\cdot NH_2 \\ | \\ NH\cdot CS_2H,NH_2\cdot CH_2\cdot CO\cdot NH_2 \end{matrix} \qquad (IV.)$$

$$\begin{matrix} CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO\cdot NH_2 \\ | \\ NH\cdot CS\cdot S\cdot CH_2Ph \end{matrix} \qquad (V.)$$

$$\begin{matrix} CH_2\cdot CO\cdot NH\cdot CH(CH_2Ph)\cdot CO_2H \\ | \\ NH\cdot CS_2H \end{matrix} \qquad (VI.)$$

glycylglycine ethyl ester hydrochloride. The reality of the dithiocarbamate intermediates postulated above was shown by the isolation of (IV), characterised as the *benzyl* ester (V), after reaction of (I) with glycine amide in ethanol.

Unlike most free peptides, the peptide ester hydrochlorides which resulted from the synthesis were highly crystalline substances with sharp melting or decomposition points, which were depressed strongly by the presence of impurities. In many cases they proved more suitable than the free peptides for analysis by partition chromatography on paper (for a review, see Consden, *Nature*, 1948, **162**, 359) because their higher R_F values led to much more satisfactory separation. This was well exemplified by the above polyglycine ester hydrochlorides and the corresponding free peptides obtained by hydrolysis. With the salts the paper chromatograms, with butanol–acetic acid as the mobile phase, showed a regular decrease in the R_F value by a factor of about $\frac{3}{4}$ for each additional glycine residue, except the first. A similar relation holds in the other polyglycine series mentioned below. By paper chromatography, it was shown that glycine was produced when (I) was warmed to 70° in water, whereas in more concentrated solution loss of carbon disulphide was accompanied also by the formation of lower polyglycines and the presence of those up to pentaglycine was demonstrated. In a similar fashion, the lower five polyglycyl-peptides of L-tyrosine, methionine, norvaline, phenylalanine, and *iso*leucine were produced by heating (I) with the appropriate amino-acid in water, or better, glacial acetic acid, and readily identified by paper chromatography. The R_F values of a number of amino-acids, glycyl-peptides, and their esters in butanol–acetic acid are collected at the end of the Experimental section.

In the case of alanine, methionine, and valine methyl esters, reaction with (I) under the standard conditions mentioned above failed to yield crystalline dipeptide ester hydrochlorides, though their formation was clearly shown by paper chromatography. This analysis also indicated the formation of several other ninhydrin-reactive substances in small amounts from such syntheses; glycine methyl ester hydrochloride and the dimeride of (I) (Part XXV, *loc. cit.*) were identified. The former was produced by reaction of (I) with the methanol used as solvent in that case, and the latter by the action of triethylamine, though the emergence of peptides in substantial yield from such reactions shows that dimerisation is slow compared with the ring-opening of (I) by amines. In such cases where the esters were not readily crystallised, the method could still be used to provide the desired peptide by hydrolysis of the crude ester and chromatography of the product on a column of cation-exchange resin (see the succeeding paper). This was illustrated by the case of glycylvaline, which was thus obtained crystalline.

Finally, it has been shown that (I) can also be utilised preparatively to give a glycyl-peptide by reaction with a free amino-acid. Thus, rapid fission occurred when (I) was shaken with a

solution of phenylalanine in two equivalents of sodium hydroxide, and on acidification a heavy yellow oil was produced which was probably the dithiocarbamic acid (VI). When this was dissolved in acetone, glycyl-DL-phenylalanine rapidly crystallised in 25% overall yield. The isolation of (VI) appeared to result from the hydrophobic character of the phenylalanine residue.

While the classical methods of peptide synthesis are too well known to require additional mention here, the use of anhydro-*N*-carboxyamino-acids bears a close relation to the present method and deserves therefore to be briefly reviewed. The first such compound, anhydro-*N*-carboxyglycine, was prepared by Leuchs (*Ber.*, 1906, **39**, 857), and some 20 additional members of the series have since been made. Interest has centred mainly on their ability to polymerise to polypeptides of high molecular weight with loss of carbon dioxide (cf., *inter alia*, Astbury, Dalgliesh, Darmon, and Sutherland, *Nature*, 1948, **162**, 596), but the formation of monomeric amides and peptides has been noted on several occasions. Thus anhydro-*N*-carboxy-*N*-phenylglycine was converted into the amide (Leuchs and Manasse, *Ber.*, 1907, **40**, 3235), anilide (Fuchs, *Ber.*, 1922, **55**, 2943), ethylamide, methylanilide, and piperidide of *N*-phenylglycine (Wessely, *Z. physiol. Chem.*, 1925, **146**, 72) and the corresponding peptides with glycine, glycine ester, and tyrosine ester (*idem*, *ibid.*). Anhydro-*N*-carboxy-β-phenylalanine gave the anilide (Curtius and Sieber, *Ber.*, 1922, **55**, 1543), amide, ethylamide, and 2 : 2-diethoxyethylamide of phenylalanine (Wessely and Sigmund, *Z. physiol. Chem.*, 1926, **157**, 91), and with glycine and glycylglycine gave the peptides phenylalanylglycine and phenylalanylglycylglycine; with tyrosine ester the peptide ester cyclised to phenylalanyltyrosine anhydride, and with aniline, phenylalanylphenylalanylanilide was isolated (*idem*, *ibid.*). By reaction of anhydro-*N*-carboxy-β-phenylalanine with aniline and methylaniline picrates, the corresponding phenylalanine amide picrates were obtained, and the same technique was also used successfully for anhydro-*N*-carboxyglycine (Wessely and John, *Monatsh.*, 1927, **48**, 11). The latter anhydride has recently been condensed with morpholine and glycine ester at a low temperature (Billimoria and Cook, *loc. cit.*). Reaction of D-anhydro-*N*-carboxyalanine with 2 equivalents of L-histidine ester in chloroform has yielded D-alanyl-L-histidine (31%), after hydrolysis (Hunt and Du Vigneaud, *J. Biol. Chem.*, 1938, **125**, 699).

In these simple cases, polymerisation is minimised by the effect of three factors, separately or in combination : (1) The loss of carbon dioxide is slow compared with the initial ring-opening reaction; this is ensured either by the effect of substituents in the oxazolid-dione ring (*e.g.*, with the derivatives of alanine and phenylalanine; cf. also 2-thio-5-thiazolidones) or by cooling (*e.g.*, with anhydrocarboxyglycine). (2) The initiating amine is usually a stronger base than that produced by loss of carbon dioxide (*e.g.*, with anhydro-*N*-carboxy-*N*-phenylglycine). (3) Where the initiating amine is a weaker base than that produced by loss of carbon dioxide, the latter is preferentially engaged by an acid, *e.g.*, picric acid, included in the medium. The use of 2-thio-5-thiazolidones described in the present paper entails the use of stable crystalline compounds which give rise to little or no complications of polymerisation.

Experimental.

Reaction of (I) *with Glycine Ethyl Ester.*—(*a*) 2-Thio-5-thiazolidone (0·68 g.) was suspended in chloroform (15 c.c.). Addition of glycine ethyl ester (1·0 g., 2 equivs.) caused dissolution with evolution of heat. Dry hydrogen chloride was passed in, the solution evaporated, and the residue treated with ethanol, to give crude glycylglycine ester hydrochloride, which had m. p. 185—186° after a further crystallisation from ethanol.

(*b*) 2-Thio-5-thiazolidone (0·66 g.) in ethanol (10 c.c.) was treated with glycine ethyl ester (1·0 g., 2 equivs.), followed after 10 minutes by 10N-ethanolic hydrogen chloride (1 c.c., 2 equivs.). Glycylglycine ethyl ester hydrochloride (0·77 g.), m. p. 155—170°, separated on cooling in ice, and was recrystallised from ethanol to give the pure compound (0·5 g.), m. p. 183—184°.

Reaction of (I) *with Glycylglycine Ethyl Ester.*—Glycylglycine ethyl ester hydrochloride (Fischer and Fourneau, *Ber.*, 1901, **34**, 2868) (8·0 g.) was suspended in chloroform and shaken rapidly with potassium hydroxide (2·5 g.) in water (3 c.c.). The chloroform was twice replaced, and the combined extracts dried (Na_2SO_4) and evaporated, to yield the free base (4·5 g.), m. p. 87—88°. The base (1·6 g., 2 equivs.) in ethanol (10 c.c.) was added to 2-thio-5-thiazolidone (0·66 g.) in ethanol (15 c.c.), and the solution acidified with 10N-ethanolic hydrogen chloride (1 c.c., 2 equivs.) after 10 minutes at room temperature. The mixture set to a solid mass of triglycine ethyl ester hydrochloride (1·2 g., 96%), m. p. 213° (decomp.) (lit., m. p. 214°), and overnight the filtrate deposited crystals of the dipeptide ester hydrochloride (0·3 g.), m. p. 180—181°, a further crop being obtained by addition of ether.

Reaction of (I) *with Glycine Ester and Triethylamine.*—2-Thio-5-thiazolidone (0·66 g.) was suspended in chloroform (10 c.c.) and cooled to 0°. A mixture of glycine ethyl ester (0·51 g., 1 equiv.) and triethylamine (0·5 g., 1 equiv.) in chloroform (2 c.c.) was added during 15 minutes, and the solution kept for a further 15 minutes in the ice bath. Acidification with 10N-ethanolic hydrogen chloride (1 c.c., 2 equivs.) then gave glycylglycine ethyl ester hydrochloride (0·57 g., 57%), m. p. 182—183°, which was collected after 2 hours at 0°. From the filtrate, overnight, a small quantity (0·05 g.) of material, m. p. 208°, was obtained.

Glycylglycine Ethyl Ester Hydrochloride.—A warm suspension of glycine ethyl ester hydrochloride (0·7 g.) in ethanol (5 c.c.) containing triethylamine (1·0 g., 2 equivs.) was added to 2-thio-5-thiazolidone (0·66 g.) in ethanol (5 c.c.), and the mixture acidified with 10N-ethanolic hydrogen chloride after 15 minutes. The product (1·17 g.), collected after 1 hour at 0°, was recrystallised from ethanol (containing free hydrogen chloride) to give somewhat impure glycylglycine ethyl ester hydrochloride (0·68 g., 70%), m. p. 162—169°.

Triglycine Ethyl Ester Hydrochloride.—2-Thio-5-thiazolidone (10 g.) was added to a mixture of glycylglycine ethyl ester hydrochloride (15 g., 1 equiv.) and triethylamine (15 g., 2 equivs.) in ethanol (150 c.c.), and the clear solution acidified with 5N-ethanolic hydrogen chloride (30 c.c., 2 equivs.). On seeding of the solution, triglycine ethyl ester hydrochloride (16·7 g., 86%) separated as a mass of plates, m. p. 213—214° (decomp.), which, recrystallised from water–acetone, had m. p. 216—217° (decomp.).

(I) (0·66 g., 1 equiv.) in 75% ethanol (10 c.c.) was treated with a solution of glycylglycine ester (0·80 g., 1 equiv.) and triethylamine (0·50 g., 1 equiv.) in 75% ethanol (5 c.c.), and acidified after a few minutes with 10N-ethanolic hydrogen chloride. Triglycine ester hydrochloride (0·8 g., 64%), m. p. 217—218° (decomp.), was then caused to separate by addition of ether. In an otherwise similar experiment, acidification was effected with 50% hydrogen bromide in acetic acid, whereupon the beautifully crystalline *triglycine ethyl ester hydrobromide* separated on scratching, and was recrystallised from ethanol, whereupon it had m. p. 191° (Found: C, 32·5; H, 5·5. $C_8H_{16}O_4N_3Br$ requires C, 32·2; H, 5·4%). The compound was soluble in methanol and insoluble in acetone.

Tetraglycine Ethyl Ester Hydrochloride.—Triglycine ethyl ester hydrochloride (12·6 g.) was shaken with triethylamine (10 g., 2 equivs.) in ethanol (400 c.c.) and filtered from a small quantity (0·8 g.) of undissolved ester. 2-Thio-5-thiazolidone (6·6 g., 1 equiv.) was added, and the solution seeded and acidified at once with ethanolic hydrogen chloride (2 equivs.), whereupon tetraglycine ethyl ester hydrochloride (12·3 g.) rapidly separated. A further 0·5 g. (total yield, 88%) of crystalline material was deposited from the filtrate. The main crop contained a small quantity of bound sulphur, which could be removed by dissolution in boiling water, filtration, and crystallisation of the "biuret base" hydrochloride as lustrous platelets, m. p. 213—214° (decomp.) (8·75 g.), by addition of ethanol containing a little free hydrogen chloride.

"Biuret base" hydrochloride prepared according to Curtius (*loc. cit.*) had m. p. 195—199° (decomp.), raised to 199—202° by one crystallisation from aqueous ethanol. With aqueous picric acid it gave a picrate, m. p. 222° (decomp.) (lit., m. p. 189°), not depressed on admixture with the picrate of "biuret base" prepared by the present method. Paper chromatography of the Curtius "biuret base" hydrochloride on Whatman No. 1 paper, with butanol–acetic acid as the mobile phase, gave three spots of R_F 0·19, 0·11, and 0·065, respectively; the first two corresponded to the known tetra- and penta-peptide ester hydrochlorides of glycine, and the third was presumed to be due to the hexapeptide.

When the above pure hydrochloride was dissolved in the minimum volume of water, addition of triethylamine caused precipitation of the free "biuret base," which crystallised well from methanol in clusters of needles or platelets. It dissolved when suspended in methanol, and the solution was treated with hydrogen bromide in acetic acid, whereafter addition of ether caused separation of *tetraglycine ethyl ester hydrobromide*, which recrystallised from ethanol as plates (Found: C, 33·8; H, 5·4. $C_{10}H_{19}O_5N_4Br$ requires C, 33·8; H, 5·4%).

Pentaglycine Ethyl Ester Hydrochloride.—Tetraglycine ethyl ester hydrochloride (5·0 g., 1 equiv.) was dissolved in water (12·5 c.c.) and solutions of triethylamine (3·2 g., 2 equivs.) in methanol (95 c.c.) and finely powdered 2-thio-5-thiazolidone (2·11 g., 1 equiv.) in methanol (65 c.c.) were rapidly added, a clear, pale pink solution resulting. Acidification with 5N-ethanolic hydrogen chloride (7 c.c.), seeding, and scratching gave the required pentapeptide ester hydrochloride (4·6 g.), m. p. 227—228° (decomp.), and a further 0·4 g. (total yield, 85%), m. p. 224° (decomp.), separated from the filtrate on storage. *Pentaglycine ethyl ester hydrochloride* recrystallised from aqueous acetone or aqueous methanol containing a little free hydrogen chloride as laths, m. p. 236—237° (decomp.) (Found: C, 39·5; H, 6·0. $C_{12}H_{22}O_6N_5Cl$ requires C, 39·2; H, 6·0%). With aqueous picric acid, it yielded a picrate, m. p. 230—231° (decomp.).

Glycyl-L-tyrosine Methyl Ester Hydrochloride.—L-Tyrosine methyl ester (0·97 g.) was boiled with chloroform (20 c.c.) and then rapidly cooled, triethylamine (0·5 g., 1 equiv.) followed by 2-thio-5-thiazolidone (0·66 g., 1 equiv.) was added, and the whole was shaken. After 4 hours, the purple solution was acidified with 10N-ethanolic hydrogen chloride (2 c.c.), and the product (0·7 g., 50%), m. p. 204° (decomp.), which was at first gummy but later crystalline, filtered off and washed with chloroform and then acetone. *Glycyl-L-tyrosine methyl ester hydrochloride* recrystallised best from methanol–ethyl acetate. It had m. p. 223 (decomp.) (Found: C, 49·4; H, 5·7; N, 9·95. $C_{12}H_{17}O_4N_2Cl$ requires C, 49·9; H, 5·9; N, 9·7%).

L-Tyrosine methyl ester (5·0 g.) was boiled with chloroform (150 c.c.) for 10 minutes, and filtered hot. To the warm filtrate was added triethylamine (3·75 g., 1·5 equivs.) and 2-thio-5-thiazolidone (5·0 g., 1·5 equivs.), and the mixture was kept overnight at room temperature. Dry hydrogen chloride was passed in until the solution was saturated, and the sticky gum which had separated rubbed with portions of chloroform to remove triethylamine hydrochloride and crystallised (3·8 g.), m. p. 217° (decomp.), by addition of acetone. The decanted chloroform slowly deposited a further 0·4 g. (total yield, 57%) of product. Recrystallisation from methanol–ethyl acetate gave the pure peptide ester hydrochloride (3·0 g.), m. p. 222—223° (decomp.).

Glycyl-DL-phenylalanine Ethyl Ester Hydrochloride.—DL-Phenylalanine methyl ester hydrochloride 1·08 g.) was dissolved in chloroform (75 c.c.) containing triethylamine (1·0 g., 2 equivs.), and the whole added to a suspension of 2-thio-5-thiazolidone (0·66 g., 1 equiv.) in chloroform (5 c.c.). Reaction appeared slower than usual, and after 30 minutes at room temperature the solution was acidified with 10N-ethanolic hydrogen chloride (2 c.c.), seeded, and cooled in ice for 4·5 hours. The glycyl-DL-phenylalanine methyl ester hydrochloride which separated (0·39 g., 30%) was washed with acetone and had m. p. 166°, undepressed on mixing with a sample made by esterifying glycyl-DL-phenylalanine (Leuchs and Suzuki, *Ber.*, 1904, **37**, 3313). The yield was not improved by addition of triethylamine to the other

two components during 1 hour, by the alternative use of pyridine, or by reaction of the thiazolidone with 2 equivs. of phenylalanine ester free base.

DL-*Phenylglycylglycylglycine Ethyl Ester Hydrochloride.*—A solution of 2-thio-4-phenyl-5-thiazolidone (0·52 g.) in ethanol (10 c.c.) was treated with a mixture of glycylglycine ethyl ester (0·4 g., 1 equiv.) and triethylamine (0·25 g., 1 equiv.) in ethanol (10 c.c.). An immediate red colour was produced, which soon faded. After 25 minutes, the solution was acidified with 10N-ethanolic hydrogen chloride (1·5 c.c.) and cooled in ice with addition of a little ether. DL-*Phenylglycylglycylglycine ethyl ester hydrochloride* (0·3 g., 37%) was collected after 30 minutes, washed with acetone, and recrystallised from methanol–ether or –acetone in small laths, m. p. 239—240° (decomp.) (Found : C, 50·3; H, 5·9. $C_{14}H_{20}O_4N_3Cl$ requires C, 51·0; H, 6·1%). The above thiazolidone afforded a crystalline triethylamine salt with the base in acetone, which regenerated the thiothiazolidone on acidification in water.

Reaction of (I) *with Glycine Amide.*—Glycine amide (1·2 g.) in ethanol (50 c.c.) was added with stirring at 0° during 45 minutes to a solution of 2-thio-5-thiazolidone (1·05 g.) in ethanol (70 c.c.). The dithiocarbamate separated as a slightly pink, deliquescent solid. A portion was shaken with benzyl chloride in ether–water (8 hours) to give N-*dithiocarbobenzyloxyglycylglycine amide*, m. p. 116—118° (hydrate ?) which recrystallised from ethyl acetate in rosettes of blades, m. p. 136° (Found : C, 49·1; H, 5·3; N, 13·7. $C_{12}H_{15}O_2N_3S_2$ requires C, 48·5; H, 5·05; N, 14·1%). When the above dithiocarbamate was dissolved in water and acidified with dilute hydrochloric acid, 2-thio-5-thiazolidone was produced in poor yield. When (I) was treated with glycine amide in acetone, the dithiocarbamate was superficially similar, but with benzyl chloride gave a low yield of a derivative, m. p. 195—196°.

Reaction of (I) *with Alanine Methyl Ester.*—Alanine methyl ester hydrochloride (1·4 g.) and triethylamine (2·02 g., 2 equivs.) were dissolved in methanol (15 c.c.), 2-thio-5-thiazolidone (1·33 g., 1 equiv.) was added, and the whole shaken. Heat was evolved and a pink solution obtained. This was acidified with 7N-methanolic hydrogen chloride (4 c.c.) and evaporated, and triethylamine hydrochloride (2·2 g.) removed by careful addition of acetone in which it was insoluble. The filtrate was analysed by paper chromatography (see final section), and shown to contain glycylalanine ester (yellow ninhydrin spot, R_F 0·37) as the main constituent, together with a little alanine ester (R_F 0·46), an unknown substance (R_F 0·55), and a glycine derivative (yellow ninhydrin spot, R_F 0·11). On storage overnight, the last substance separated in small amount, and had m. p. 195 °(decomp.); it was sparingly soluble in ethanol. The filtrate was evaporated, the soluble hydrochlorides were extracted into water, and the bases liberated with potassium carbonate. Treatment with ethereal picric acid gave a small quantity of picrate, m. p. 93—125°, which could be recrystallised from ethanol and on chromatography on paper separated into a picric acid spot and a ninhydrin-reactive spot (R_F 0·55). It would thus appear to be the picrate of the unknown compound above.

Reaction of (I) *with Methionine Methyl Ester.*—2-Thio-5-thiazolidone (0·33 g.) was added to a mixture of methionine methyl ester hydrochloride (0·50 g., 1 equiv.) and triethylamine (0·50 g., 2 equivs.) in methanol (5 c.c.) and the whole shaken. After 20 minutes, 7N-methanolic hydrogen chloride (1 c.c.) was added, and the mixture evaporated and treated twice with acetone to give crops (total, 0·55 g.) of triethylamine hydrochloride. Paper chromatography of the filtrate showed it to be a mixture of the required glycyl-DL-methionine methyl ester hydrochloride (R_F 0·55) with unchanged methionine ester hydrochloride (R_F 0·75), 2-thio-5-thiazolidone dimer (reddish-purple spot, R_F 0·39), and glycine. Both the thiazolidone and diaminoacetoacetic acid to which it readily gives rise yield a characteristic reddish-purple ninhydrin spot at R_F 0·27 (glycine, 0·065), preceded by a " tail " in the former instance.

Reaction of (I) *with Valine Methyl Ester.*—2-Thio-5-thiazolidone (0·67 g.) was added to a mixture of valine methyl ester hydrochloride (0·84 g., 1 equiv.) and triethylamine (1·02 g., 2 equivs.) in dry methanol (10 c.c.) and the whole shaken. After 15—30 minutes, the reddish-brown solution was acidified with 7N-methanolic hydrogen chloride (2 c.c.) and evaporated to a gum, from which triethylamine hydrochloride was removed by two treatments with acetone. The filtrate contained largely glycyl-DL-valine methyl ester hydrochloride (R_F 0·66), together with valine ester (R_F 0·76) and a small amount of a substance having R_F 0·36 (yellow spot), together with a trace of glycine (R_F 0·10). On evaporation and treatment with ethyl acetate, the substance having R_F 0·36 separated; recrystallised from ethanol–ether, it had m. p. 178° (decomp.); analysis showed it to be glycine methyl ester hydrochloride (Found : C, 29·3; H, 6·4; N, 11·0. Calc. for $C_3H_8O_2NCl$: C, 28·7; H, 6·4; N, 11·15%). The filtrate, which darkened when kept overnight, did not yield the required peptide in crystalline condition.

The above synthesis was also carried out in chloroform solution, and in this case paper-chromatographic analysis of the product, after removal of the triethylamine hydrochloride, showed that glycine methyl ester was absent but that two other impurities (R_F 0·40 and 0·46) were present. The former had the characteristic reddish-purple ninhydrin reaction of the dimer of (I), and the latter was a glycyl derivative (yellow ninhydrin spot) the constitution of which is unknown. The oily glycylvaline ester hydrochlorides from this and the preceding experiment were combined and hydrolysed with 2N-sodium hydroxide. The solution was acidified with acetic acid and evaporated to dryness, whereafter treatment with ethanol caused separation of sodium chloride, which was removed. The filtrate was evaporated, dissolved in water, and chromatographed on a column of " Zeokarb 215 " as described in the succeeding paper. Crystalline glycyl-DL-valine, m. p. 239° (decomp.), chromatographically pure, was obtained in rather poor yield.

Reaction of (I) *with Phenylalanine.*—DL-Phenylalanine (0·83 g.) was dissolved in N-sodium hydroxide (10 c.c., 2 equivs.), 2-thio-5-thiazolidone (0·67 g., 1 equiv.) added, and the whole shaken to give a clear yellow solution. After 20 minutes this was acidified with concentrated hydrochloric acid (2·0 c.c.), and the heavy yellow oil which separated was removed and washed with water. Dissolution in acetone and seeding caused glycyl-DL-phenylalanine (0·28 g., 25%), m. p. 266° (decomp.), to separate rapidly; it recrystallised from aqueous ethanol in clusters of needles, m. p. 267° (decomp.). Similarly, when glycyl-DL-phenylalanine (0·96 g.) was shaken with a solution of potassium hydroxide (0·48 g.) in water (3·5 c.c.) and carbon disulphide (0·4 g.) for 2·2 hours, and the resulting clear reddish-orange solution acidified with concentrated hydrochloric acid, a heavy yellow oil was produced, which yielded glycylphenylalanine when dissolved in acetone.

Paper Chromatography.—For this work, unidimensional, descending chromatograms were run on Whatman No. 1 paper, with butanol–acetic acid (BuOH : AcOH : H_2O = 4 : 1 : 5) as the mobile phase. This solvent gave sharper spots and a cleaner paper, and was more pleasant to handle than phenol. Temperatures varied from about 15° to 25° on different occasions. The R_F values varied considerably with different papers, temperatures, etc., though their relative values remained more constant. Glycine was therefore arbitrarily selected as standard with R_F 0·10, and run with every chromatogram, the observed R_F values of other substances being computed proportionally. This correction was of less value with substances of high R_F values, which remained more constant under different experimental conditions and was therefore not applied in such cases (see tables). All spots were yellow initially with ninhydrin, but changed to the usual purple colour after keeping for some hours. In practice all the various crops of crystals, filtrates, etc., were analysed chromatographically though only certain significant determinations are reported. Values found are given in the annexed tables.

R_F *Values in butanol–acetic acid.*

	Free acid.	Me ester hydrochloride.	Et ester hydrochloride.	Amide.		Free acid.	Me ester hydrochloride.	Et ester hydrochloride.
Glycine	0·10	0·36	0·37	—	Glycylglycine	0·096	0·25	0·335
Alanine	0·18	0·46	—	—	Triglycine	0·078	—	0·25
Valine.....	0·42	0·53	—	—	Tetraglycine	0·053	—	0·18
Leucine	0·57	—	—	—	Pentaglycine	0·033	—	0·12
Norleucine ...	0·96	—	—	0·43	Glycylalanine	0·37	0·26	—
Phenylalanine	0·50	0·66	—	—	Glycylvaline	0·37	0·46	—
Methionine ...	0·33	0·52	—	—	Glycylphenylalanine	0·36	0·52	—
Tyrosine	0·54	0·50	—	0·31				
Glutamic acid	0·14	—	0·69 [a]	—				
Arginine	0·07	—	—	—				

Diglycylphenylalanine ...	0·30	Glycyl-leucine	0·42	Glycylnorleucine	0·38
Triglycylphenylalanine ...	0·23	Diglycyl-leucine	0·33	Diglycylnorleucine	0·30
Tetraglycylphenylalanine	0·18	Triglycyl-leucine	0·25	Triglycylnorleucine	0·23
Pentaglycylphenylalanine	0·14	Tetraglycyl-leucine	0·21	Tetraglycylnorleucine ...	0·17
Hexaglycylphenylalanine	0·11	Pentaglycyl-leucine.....	0·155	Pentaglycylnorleucine ...	0·12

Glycylmethionine methyl ester hydrochloride	0·38	Glycyltyrosine methyl ester hydrochloride ...	0·48	Polymer (MeOH, EtOH, pyridine) of (I)	0·52
		Diethyl ester hydrochloride.			

We express our gratitude to Sir Ian Heilbron, D.S.O., F.R.S., for his very helpful interest, and to the Department of Scientific and Industrial Research for a Senior Research Award to one of us (A. L. L.).

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
S. KENSINGTON, LONDON, S.W.7.

[*Received, November 18th*, 1949.]